The chelons of the present invention bind a variety of transition metals with relatively high efficiency (affinity). In these experiments we exposed fully reduced chelon (SEQ ID NO: 4) to a 2-fold molar excess of the indicated metals in the presence of a large excess of competing thiol (5 mM 2-mercaptoethanol) and then dialyzed the reactions extensively against a buffered thiol and determined the metal content by inductively coupled plasma-mass spectrometry (ICP-MS). The MBD binds cadmium, cobalt, copper, lead, nickel, and zinc with an affinity equivalent to that for Hg(II), i.e. one metal atom per protein molecule. Interestingly, it does not bind two other "thiophilic metals" iron and arsenite, A(III).

The natural MerR protein has an additional property of interest: it undergoes a conformation (allosteric) change when metal ion binds to it [Heltzel et al. (1990) Biochemistry 29, 9572-9584]. In the intact protein this change is transduced from the metal binding domain to the DNA binding domain and results in underwinding of the DNA to foster transcription initiation. The MerR binding domain is the first functional metalloprotein subdomain which is sufficiently small and stable for use outside of the cellular environment [Zeng et al. (1998) supra].

The present inventors discovered that the chelon protein of SEQ ID NO:4 binds the same ions as the wild-type MerR protein. While the wild type MerR protein binds certain ions other than mercuric ions, those other ions do not induce transcription of the *mer* operon in vivo or in vitro.

Because most proteins require that all of their subdomains fold together with each other in order to achieve a stable tertiary structure and avoid degradation, it was not obvious that any single subdomain of MerR could be produced as a stable functional subdomain within the cells. The present findings indicate that the tendency of the MerR metal binding domain to fold into stable, functional polypeptide structure is strong. It was also not obvious that the unnatural direct linkage of two of these domains would yield a stable protein, much less one which retained the metal binding properties of the natural MerR dimer.

Building both halves of the metal binding domain into a single polypeptide eliminates the problem of achieving sufficiently high protein concentrations assure the optimum formation of dimers; i.e., the two halves of the binding domain don't need to "find" each other because they are covalently joined by a linker peptide region. Even with an affinity tag the chelon derivative is only slightly larger than a typical metallothionein and it will be much less costly to the cell to produce than the full length MerR protein. The stability of the folded form of the metal binding domain and the allosteric change which the MerR metal binding domain undergoes allow its use outside the cell in both metal detection and metal sequestration strategies.

In metal sequestration application of the present chelon technology, the cells in which the chelon is expressed contain strongly bound metal ions which will not significantly exchange with extracellular metal ions. Due to the high affinity binding, those cells are significantly more resistant to the heavy metal ions than comparison cells which do not express the chelon protein(s). Bacterial cells engineered to express at least one chelon coding sequence are useful in bioremediation of contamination sites. Colonizing the intestinal tract with nonpathogenic nontoxigenic bacteria engineered to express at least one chelon reduces enterohepatic recycling of mercury and makes elimination more rapid and more efficient. Most of the ingested and inhaled mercury is processed through the intestinal tract of primates, and colonization with chelon-producing microbes reduces uptake of heavy metal ions into circulation.

Purified chelon proteins can be used isolated away from the cellular environment. Present water treatment resins have relatively low affinities for toxic metals when nontoxic metal are in comparatively high concentration and compete for binding to the resin. A resin which "ignores" nontoxic metals and binds only a target metal of interest (especially mercury or cadmium cations) can augment existing water treatment regiments. Immobilizing a chelon of the present invention to a solid support allows for the design of water treatment resins useful in removing mercury and/or cadmium, depending on the choice of the particular chelon or a combination of chelons. Suitable solid supports include, without limitation, latex particles, glass beads, hydrogels, polystyrene and liposomes. The particles or beads can be fabricated from a variety of materials and can have any shape, including a spherical shape. The chelon(s)

can be on the surfaces of the beads or particles, or they can be within porous beads or particles. The beads can have added surface groups to act as spacers to improve access to the metal ion binding sites of the attached chelon proteins or to facilitate attachment of the chelon molecules, for example carboxyl groups on latex or amino modifications on polystyrene. Polymers with free amino groups that are useful in conjunction with gelling agents include, without limitation, alginate amine, chitosan, pectin, and polyethylene imine. Additionally, affixing the chelon(s) to microspheres with selective permeability allows it to access mercury attached to low molecular weight thiols in the environment while protecting the chelon protein from degradative enzymes in the environment. Magnetic versions of such microspheres can be disseminated in the soil. After a time sufficient to allow the microspheres to become saturated with toxic metal ions, the beads are recovered via standard magnetic recovery techniques.

MerR or chelon proteins can be readily engineered to facilitate purification and/or immobilization to a solid support of choice. For example, a stretch of 6-8 histidines can be engineered through mutagenic polymerase chain reaction or other recombinant DNA technology to allow purification of expressed recombinant protein over an nitrilotriacetic acid (NTA) column using commercially available materials. Other oligopeptide "tags" which can be fused to a protein of interest by such techniques include, without limitation, strep-tag (Sigma-Genosys, The Woodlands, TX) which directs binding to streptavidin or its derivative streptactin (Sigma-Genosys); a glutathione-S-transferase gene fusion system which directs binding to glutathione coupled to a solid support (Amersham Pharmacia Biotech, Uppsala, Sweden); a calmodulin-binding peptide fusion system which allows purification using a calmodulin resin (Stratagene, La Jolla, CA); a maltose binding protein fusion system allowing binding to an amylose resin (New England Biolabs, Beverly, MA); and an oligo-histidine fusion peptide system which allows purification using a Ni^{2+}-NTA column (Qiagen, Valencia, CA).

The range over which the chelons of the present invention act to bind mercury (and cadmium in the case of the mercury and cadmium specific chelon) extend from as low as 10^{-9}